

U.S.S.N. 09/14/220
A 102

130
120
110
100
90
80
70
60
50
40
30
20
10
0

TNQRSPPPGERTRGRQP GDYDDDRQPRREEGGRRQPREREEDW.RQPR
TNQRSPPPGERTR
QRSPPPGERTR
SPPGERTRGR
PGERTRGRQP
ERTRGRQPGD
TRGRQP GDYD
GRQP GDYDDD
QP GDYDDDR
GDYDDDRRQ
YDDDRRQPRR
DDR RQPRREE
RR QPRREEGG
QPRREEGGRW
RREEEGGRWGP
EEGGGRWGPAG
GRWGPAGPR
AGPRERERE
PREREREEDW
EREREEEDWRQ
EREEDWRQPR

F/G 1

U.S.S.N. 09/141, 220
45 102

55 6 65 7 75
 | | | |
 DSYERD**PYSPSQDPYS**SPSPYDR
 DSYERD**PYSP**
 YERD**PYSPSQDPYS**
 RDPYSPSQDPYSPS
 PYSPSQDPYS
 SPSQDPYS
 SQDPYS
 DPYS

FIG. 2

299 | 321 |
EEE YDEDE EYE YDEEDRR RGRGSR
1. EEE YDEDE EYE YDEED
2. EYDEDE EYE YDEEDRR
3. DEDE EYE YDEEDRR RG
4. DEYE YDEEDRR RGRG
5. YE YDEEDRR RGRGSR
6. YDEEDRR RGRGSGRGR

FIG. 3

H.S.S.N. 09/14/220
15102

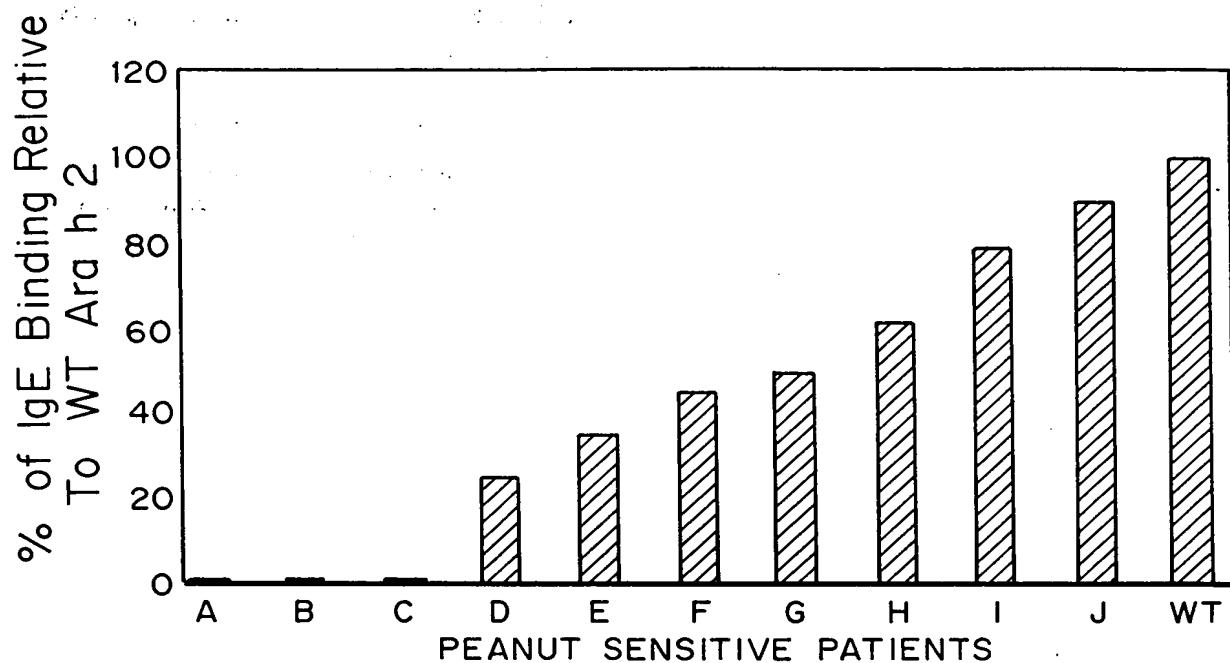


FIG. 4

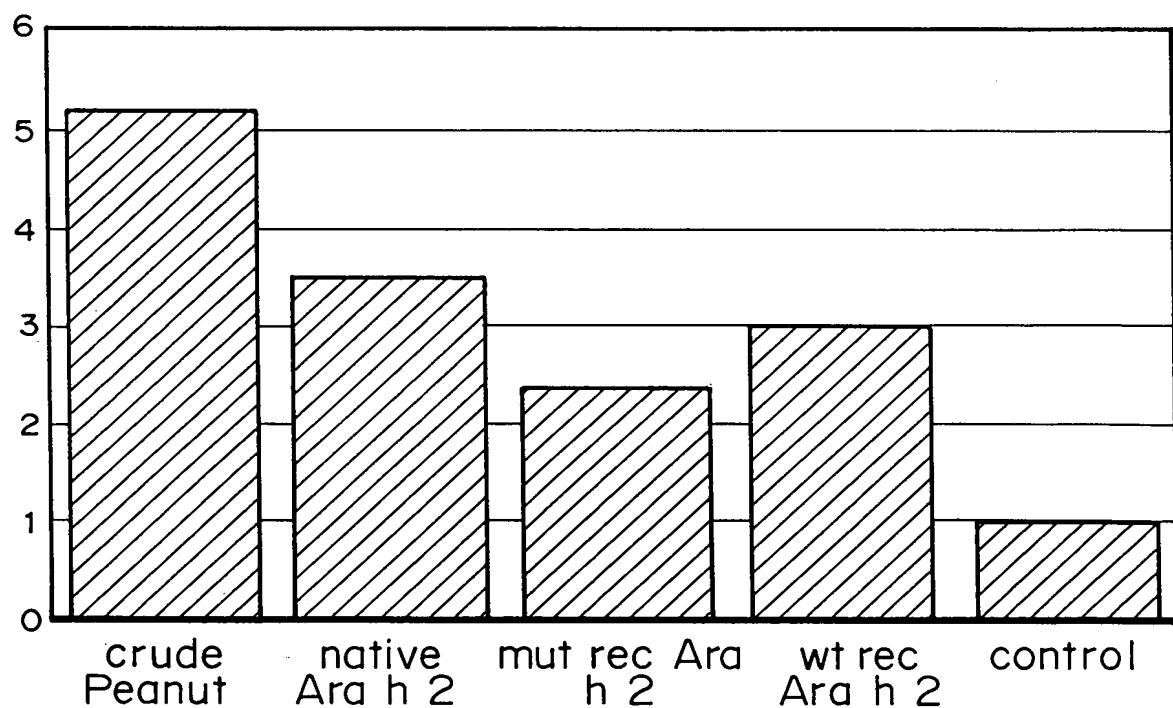


FIG. 5

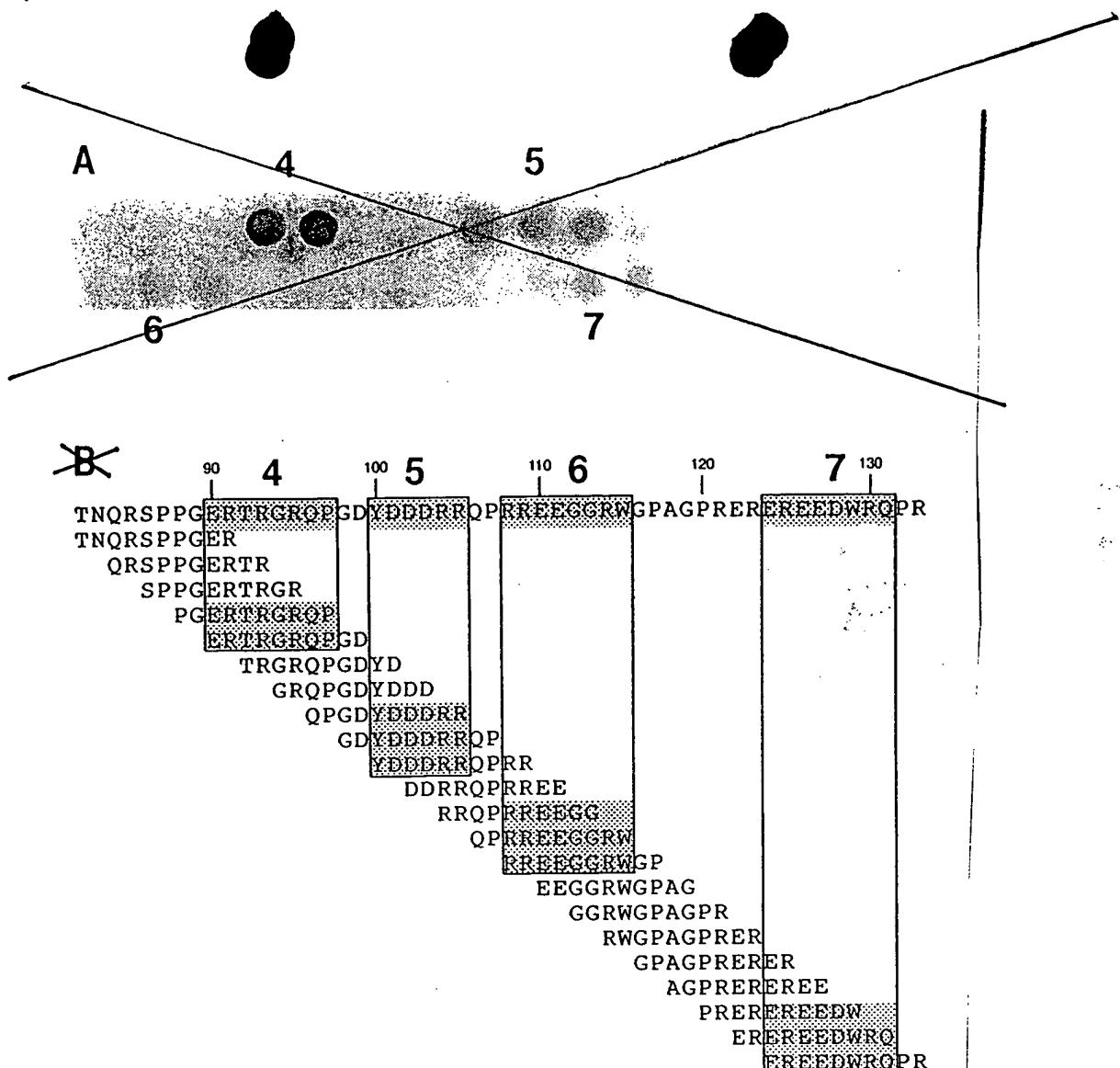


Fig. 1. Core IgE-binding epitopes identified on the Ara h 1 allergen. Epitope analysis was performed on the IgE-binding sites by synthesizing 10 amino-acid long peptides offset by two amino acids. These peptides were then probed with the 15 patient serum pool. (A) The peptide analysis of Ara h 1 amino-acid residues 82-133. This region contains peptides 4, 5, 6 and 7 identified in Table 1. (B) The amino acid sequence of this region.

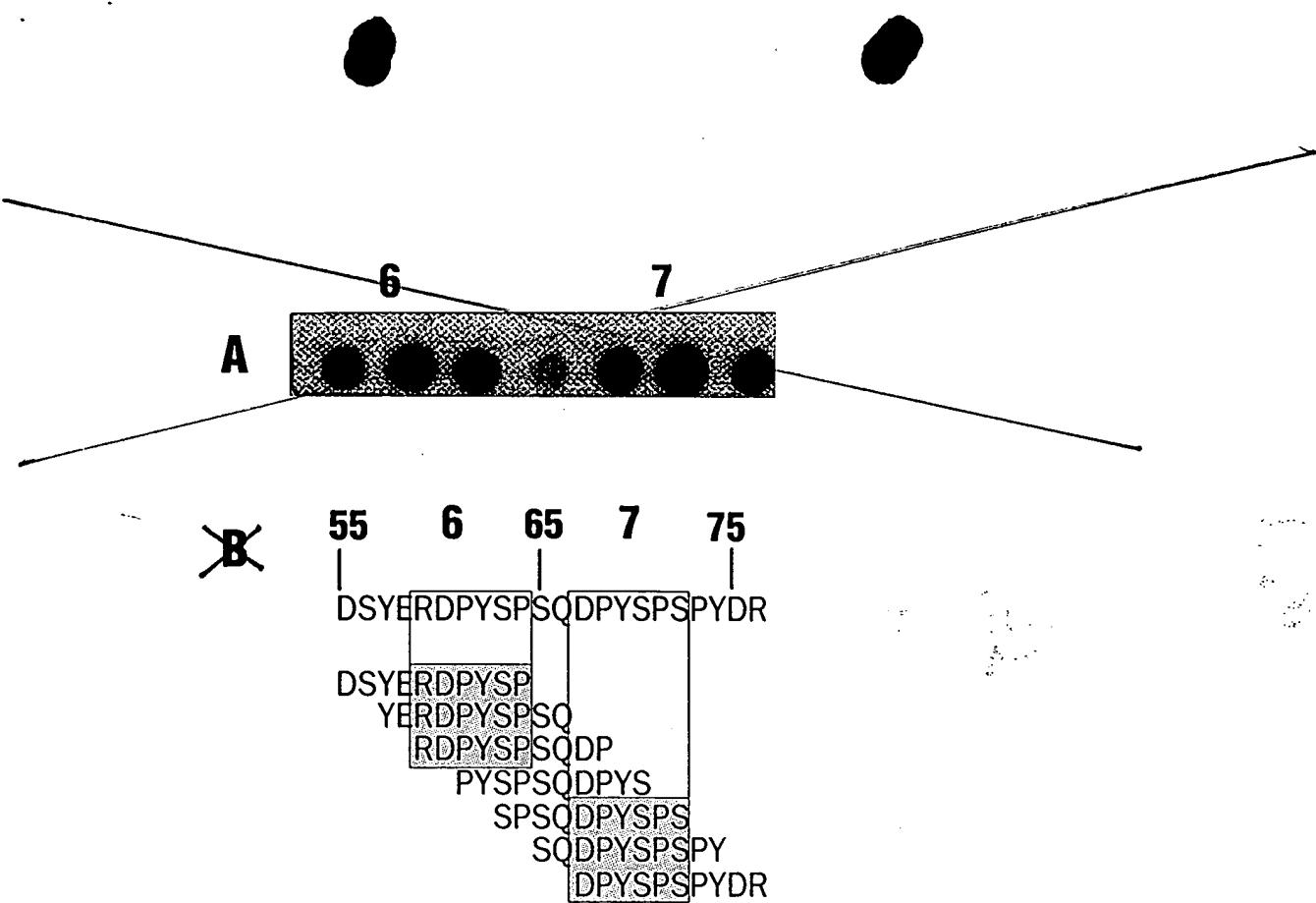


Fig. 2. Core IgE-binding epitopes identified on the Ara h 2 allergen. Epitopes analysis was performed on the IgE-binding sites by synthesizing 10 amino acid long peptides offset by two amino acids. These peptides were then probed with the 15-patient serum pool. (A) The peptide analysis of Ara h 2 amino acid residues 55-76. This region contains peptides 6 and 7 identified in Table 2. (B) The amino acid sequence of this region.

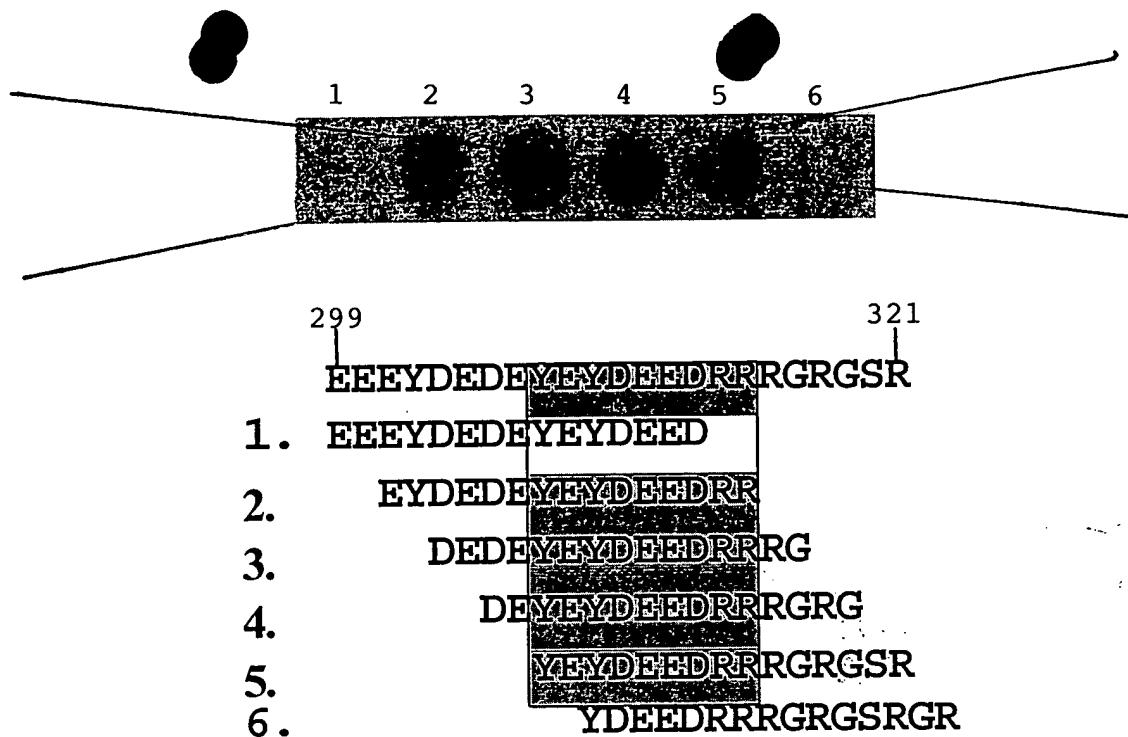


Fig. 3. Core IgE-binding epitopes identified on the Ara h 3 allergen. Epitopes analysis was performed on the IgE-binding sites by synthesizing 15 amino acid-long peptides offset by two amino acids. These peptides were then probed with the 15 patient serum pool. (A) The peptide analysis of Ara h 3 amino acid residues 299-321. This region contains peptides 4 identified in Table 3. (B) The amino acid sequence of this region.

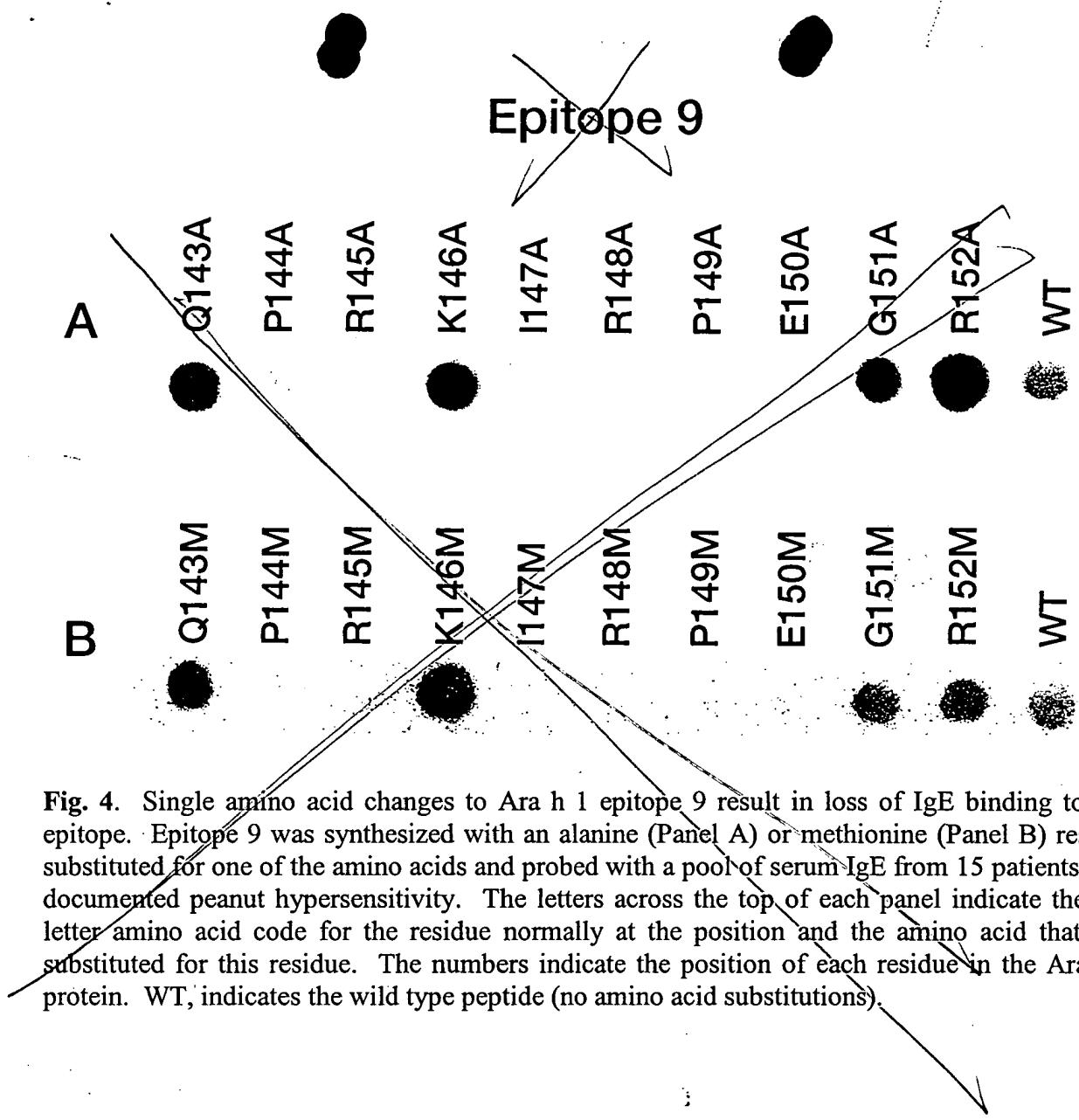


Fig. 4. Single amino acid changes to Ara h 1 epitope 9 result in loss of IgE binding to this epitope. Epitope 9 was synthesized with an alanine (Panel A) or methionine (Panel B) residue substituted for one of the amino acids and probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity. The letters across the top of each panel indicate the one letter amino acid code for the residue normally at the position and the amino acid that was substituted for this residue. The numbers indicate the position of each residue in the Ara h 1 protein. WT, indicates the wild type peptide (no amino acid substitutions).

~~Fig. 5. A modified Ara h 2 protein binds less IgE than the wild type Ara h 2 allergen. The Ara h 2 amino acid sequence is represented as the single letter amino acid code. Alanine was substituted for the wild type residue at the position indicated (Upper Panel). Relative amounts of IgE binding to modified Ara h 2 proteins were quantitated and expressed as a percentage of binding to the wild type allergen (Middle Panel). Immunoblot analysis of IgG binding to wild type and modified Ara h 2 proteins showing that this immunoglobulin still binds the modified protein (Lower Panel).~~

Ara h 2 Amino Acid Sequence

L T I L V A L A F L L A A H A S A R Q Q W E L Q G D R R C Q S Q L E R A - 37

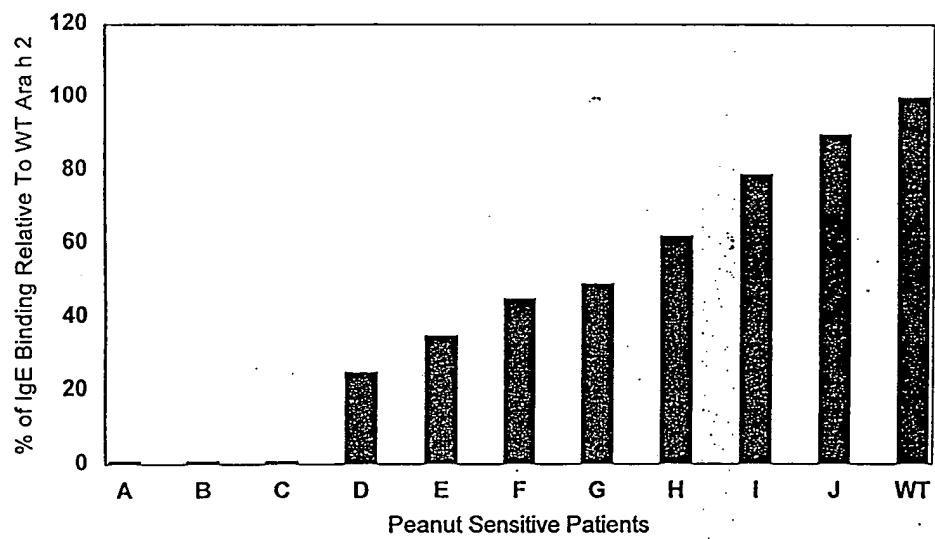
N L R P C E Q H L M Q K I Q R D E D S Y E R D P Y S P S Q D P Y S P S P Y - 74

D R R G A G G S S Q H Q E R C C N E L N E F E N N Q R C M C E A L Q Q I M E - 111

N Q S D R L Q G R Q Q E Q Q F K R E L R N L P Q Q C G L R A P Q R C D L D - 148

V E S G G R D R Y - 157

IgE Binding To Mutant Ara h 2



WT MUT WT MUT
Individual Pool

T-Cell Proliferation Assay

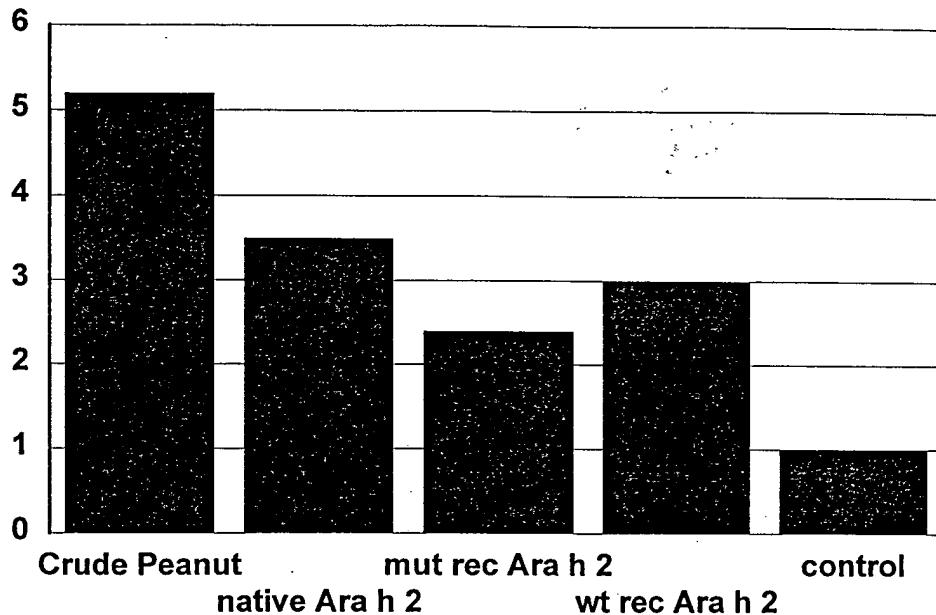


Fig. 6. The peripheral blood lymphocytes (PBLs) of peanut sensitive patients were isolated from whole blood using standard procedures of ficoll hypaque underlay. Cells were washed and suspended in media at a concentration of 4×10^6 cell/ml. One ml aliquots were placed in 24 well tissue culture plates and stimulated with 50 mg/ml of crude peanut extract (CPE) in order to establish peanut-specific cell lines. For the proliferation assays, T-cell lines at 2×10^5 cells/well were stimulated with either media alone (control), CPE (50 μ g/ml), native Ara h 2 (10 μ g/ml), mutant recombinant Ara h 2 (10 μ g/ml), or wild type recombinant Ara h 2 (10 μ g/ml) and allowed to proliferate for 6 days. On the last day the cells were incubated with [³H]-thymidine (1 mCi/well) for 6-8 hours and then harvested onto glass fiber filters (Packard, Meriden, CT). T-cell proliferation was estimated by quantitating the amount of [³H]-thymidine incorporation into cellular DNA. [³H]-thymidine incorporation is expressed as a stimulation index which is defined as the fold-increase above media-treated cells.